a1

20. (Amended) A method for producing a thermostable cellulase comprising maintaining the host cell of Claim 19 under conditions suitable for expression of said nucleic acid construct, whereby said thermostable cellulase is produced.

Please add new Claim 30.

a2

30. (New) An isolated nucleic acid molecule encoding a polypeptide, wherein the polypeptide is more soluble and has a specific activity of at least two times greater than the specific activity of the full length native counterpart comprising SEQ ID NO: 2 and said nucleic acid is truncated such that one or more of the amino acid residues corresponding to position one to about position 40 in the corresponding full length enzyme are deleted in the polypeptide encoded by said nucleic acid molecule.

REMARKS

Applicants have amended Claims 1-20. Claims 1 and 15 have been amended to correct typographical errors. Claims 1, 2, 4, 7, 10-12 and 14-17 have been amended to describe the nucleic acid as a "nucleic acid molecule", support for this amendment can be found on page 11, lines 13-17. Claims 2, 3, 5, 6, 8, 9, 12, 13 and 17-20 have been amended to recite an "nucleic acid construct", support for this amendment can be found on page 11, lines 13-15. Claims 1, 4, 7, 11 and 15 have been amended to recite a polypeptide having "improved" thermostable cellulase activity. Support for these amendments can be found in the Specification on page 3, lines 4-5. Claims 1 and 15 have been amended to include the phrase "at least 85 % identity compared to SEQ ID NO: 2". Support for this amendment can be found in the Specification on page 8, lines 16-27. New Claim 30 has been added. Support for Claim 30 can be found throughout the Specification, in particular, on page 5, lines 23-27. No new matter is added.

Claims 23-29, drawn to a non-elected invention, have been cancelled. Applicants reserve the right to file a continuing application or take such other appropriate action as deemed necessary to protect the non-elected invention. Applicants do not hereby abandon or waive any rights in the non-elected invention.

Claim Objections

Claims 1 and 15 have been amended to correct spelling errors noted by the Examiner. Claims 1, 4, 7, 10, 11, 14, 15 and 16 have been amended to clearly recite that the isolated nucleic acid is an "isolated nucleic acid molecule".

Rejection of Claims 2-3, 5-6, 8-9, 12-13 and 17-22 under 35 U.S.C. §112, Second Paragraph

Claims 2-3, 5-6, 8-9, 12-13 and 17-22 are rejected under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject mater which the applicant regards as the invention.

Applicants have amended the claims to recite "a nucleic acid construct". As amended the claims are clear and definite. Applicants request reconsideration and withdrawal of the rejection.

Rejection of Claims 1-3, 15-20 U.S.C. §112, First Paragraph

Claims 1-3 and 15-20 are rejected under 35 U.S.C. §112, first paragraph, because the Examiner states that the specification does not reasonably provide enablement for any variant DNA sequence from any source encoding a variant glycosyl hydrolase of family 12, and does not enable a person skilled in the art to which it pertains to use the invention commensurate in scope with these claims. Applicants respectfully disagree.

The Claims have been amended to recite structural and functional attributes that characterize the genus of variants claimed. For example, in some embodiments, the variants must have at least 85% identity to SEQ ID NO: 2. In other embodiments, the variants have been structurally described by the particular sequences recited. This criteria sets forth a **finite** number of possibilities that must be tested. In view of these points, the amended claims are not overly broad and considerable direction is provided.

The Specification specifically teaches regions of the DNA sequence which may be modified without effecting the enzymatic activity (See Specification, page 28, line 18 to page 30, line 5). The Specification teaches removal of one or more amino acid residues that are not part of the catalytic domain (*e.g.*, amino acids in the amino terminal hydrophobic domain and/or linker moiety). From this teaching, there are parts of the sequence that are clearly more tolerant to changes in sequence than others. Applicants' Claims recite that the cellulase activity of

resultant polypeptides encoded by the nucleic acids must be "improved" compared to SEQ ID NO: 2. Thus, Applicants have provided a measure to determine the tolerance of the changes that would be acceptable.

Using the teachings and sequences described in the Specification as a guide, one skilled in the art would certainly be capable of making additions, deletions and substitutions within the provided sequence framework. Additionally, a method to test the resultant variants in the enzymatic assay is detailed in the Exemplification. Therefore, the Specification details the necessary knowledge to determine a variant's inclusion in the genus. Such experimentation is routinely undertaken in the art and would not be considered undue.

Even though some experimentation is needed, the courts have held that "[e]nablement is not precluded by the necessity for some experimentation such as routine screening." *In re Wands*, 8 USPQ2d 1400, 1404 (Fed.Cir. 1988). "[A] considerable amount of experimentation is permissibly if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed."... "Enablement does not require absolute predictability, but that the person of ordinary skill in the art be able to practice the invention without undue experimentation." *Id.*
The experimentation needed to determine the variants encompassed by the Claims is clearly routine, and the Specification provides the necessary guidance. The Specification discloses methods suitable for preparing truncated nucleic acids encoding thermostable cellulases belonging to the glycosyl hydrolase family 12 and polypeptides encoding such thermostable cellulases and for determining the enzymatic activity of the resultant polypeptides.

In view of the above, Applicants submit that the claimed invention is enabled, and respectfully request reconsideration and withdrawal of the rejection.

Rejection of Claims 1-3 and 15-20 under 35 U.S.C. §112, First Paragraph

Claims 1-3 and 15-20 are rejected under 35 U.S.C. §112, first paragraph, as containing subject matter which was not described in the Specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors at the time the application was filed had possession of the claimed invention. The Examiner states that the Specification does not contain any disclosure of the structure and function of all variant DNA sequences and that the

genus of DNAs is a large variable genus with different structures and the potentiality of encoding many different proteins. The Examiner further directs Applicants to the revised guidelines concerning compliance with the Written Description Requirements of U.S.C. §112, First Paragraph.

The written description requirement is satisfied when the specification describes the invention in sufficient detail so that one skilled in the art can reasonably conclude that the inventor had possession of the claimed invention. *Vas-Cath, Inc. v. Mahurhar*, 19 U.S.P.Q.2d 1111 (Fed Cir. 1991). The Guidelines specifically provide that when a genus is claimed, the specification should provide common attributes shared by members of the genus to distinguish compounds in the genus from other materials. The training materials include examples which illustrate the application of substantive law to particular facts. See Examples 13 and 14.

Applicants have amended the claims to refer to structural attributes comprising nucleic acid molecules encoding a polypeptide of a glycosyl hydrolase of family 12, specific nucleotide sequences (SEQ ID NO: 3 and specific nucleotides of SEQ ID NO: 3) or amino acid sequences (SEQ ID NO: 2 and specific sequences of SEQ ID NO: 2), thus setting forth common attributes and characteristics of the genus. Thermostable cellulases of glycosyl hydrolase family 12 have common sequence identity in their catalytic domains (See Figure 1 of Halldorsdottir *et al.)*. The family also shares common enzymatic characteristics such as thermostability and substrate specificity.

Applicants discovered that the N-terminal hydrophobic region and/or linker moiety of the full-length cellulase contributed to the cytotoxicity and aggregation of the native enzyme. A problem and negative feature of the gene that resulted in low yields of recombinant enzyme. Applicants solved this problem by removing amino acids from the N-terminal end of the enzyme and were able to increase production yields of the variant enzymes. Additionally, Applicants demonstrate that the removal (*i.e.,* truncation) of one or more amino acids from the N-terminal hydrophobic region and/or linker moiety produced variants of thermostable cellulases with improved activity, stability and solubility over the native or full-length cellulase. The improved activity of two of these variants are shown in Table 2 of the Specification. One skilled in the art upon reading the teachings of the Specification would recognize the problem associated with this negative feature of the gene and the solution Applicants disclose. It would then be apparent that

enzymes that belong to this family of enzymes who possess this problem can be improved with truncation. Applicants teach the methods for the preparation of truncated enzymes and an enzymatic assay to assess if the truncation worked. Based on the teachings of the Specification, one skilled in the art can clearly ascertain the members of the genus Applicants are claiming.

In view of these features, Applicants have satisfied the written description requirement and respectfully request reconsideration and withdrawal of the rejection.

Rejection of Claims 1-22 under 35 U.S.C. §103(a)

Claims 1-22 stand rejected under 35 U.S.C. §103(a) as being obvious over Halldorsdottir *et al.* (*Appl. Mocrobiol. Biotechnol.*, 49:277-284 (1998)), the sequence alignment of SEQ ID NO: 2 and Gene Accession No. RMU72637, Gilkes *et al.* (*Microbiol. Rev.*, 55(2):303 (1991)), and the high level of knowledge existing in the art. Applicants respectfully disagree.

Applicants' invention pertains to the unexpected discovery that removal of the N-terminal hydrophobic region and/or the linker moiety of glycosyl hydrolases of family 12 yielded polypeptides of thermostable cellulases with superior catalytic and physical properties when compared to the native full-length polypeptide. These variant polypeptides can now be produced efficiently in recombinant organisms with superior properties, such as increased stability, solubility and specific activity. See Specification, page 5, lines 13-20. The variant polypeptides have pH optima and substrate specificities similar to full-length polypeptides but their specific activities were improved - surprisingly three-fold higher than the specific activity of the full length enzyme. The variant polypeptides were also shown to be more stable than the full length enzyme. These unexpected superior characteristics allow for increased versatility in commercial applications. For example, the improvement in specific activity allows one to reduce the amounts of enzyme needed in reactions, thereby providing cost advantages.

Halldorsdottir *et al.* teach cloning, sequencing and overexpression of a *Rhodothermus marinus* gene encoding a thermostable cellulase of glycosyl hydrolase family 12. Furthermore, vectors, host cells and methods of producing the cellulase in the host cells are provided. However, there is no teaching or suggestion in Halldordottir *et al.* of the benefit of N-terminal truncations of the cellulases. Therefore, this reference only teaches characterization and methods of producing the wild-type form of the cellulase from *R. marinus.*

Applicants did not merely retain the catalytic activity with the variants produced from less than the full length gene but showed increased catalytic activity. Applicants demonstrated that the N-terminal end was detrimental to the cells and further that removal of amino acids at the N-terminal end produced variants with superior properties, including over a three-fold increase in catalytic activity.

In the Exemplification of the instant application, the full length enzyme was shown to be inefficiently expressed. The *E. coli* cells lysed soon after induction of the full length enzyme indicating that the full length enzyme was cytotoxic. Further, the full length enzyme had reduced stability losing activity when stored over a period of time. Additionally, the full length enzyme was shown to aggregate and displayed poor thermostablity when the enzyme was heat treated at 65°C, which is the physiological temperature of the host organism *R. marinus*. See Specification, page 5, lines 2-4. Applicants discovered a way to increase expression, decrease aggregation, increase enzymatic activity and increase stability of the polypeptides by removal of amino acids from the N-terminal end of the polypeptide. The polypeptides of the invention possess improved characteristics, such as increased stability (*e.g.*, thermal stability, detergent stability), increased solubility in aqueous solvents, increased catalytic activity (*e.g.*, specific activity, catalytic rate) and/or reduced cytotoxicity relative to the native or full-length thermostable cellulase, but retain the substrate specificity of the native or full-length cellulase. See Specification page 8, lines 19-26. These statements are further supported by the "Declaration Pursuant to 37 C.F.R. § 1.132 by Gudmundur O. Hreggvidsson, Ph.D.," filed concurrently herewith. These beneficial properties and increased yield of the thermostable cellulase are not taught or suggested in Halldorsdottir *et al.* There is no suggestion in the Halldorsdottir *et al.* reference that a variant of less than the complete gene would yield an enzyme with any of the superior properties discussed above.

Like Halldorsdottir *et al.*, Gilkes *et al.* do not provide the necessary motivation or teaching to produce a functional truncated enzyme that is better than the native enzyme. Gilkes *et al.* generically teach the structural elements in β-1,4-glycanases using sequence comparison, functional comparison and relationship between enzyme families. Table 5 in Gilkes *et al.* describes only one organism of the same family of H2 hydrolases such as Applicants' hydrolases and does not describe the actual domains of this family. Specifically, Gilkes *et al.* do not suggest

that producing variants of less than the full length enzyme would produce the superior properties discovered by Applicants. At best, this article demonstrates that the catalytic domains and cellulose-binding domains **merely retain** their function when separated by proteolysis. Gilkes *et al.* do not teach the variant combinations of a catalytic domain, linker and signal peptide that could be combined to produce an improved cellulase, such as Applicants teach. The reference does not teach a specific combination of the domains to produce an enzyme with superior qualities. In fact, the reference does not indicate the role of signal peptides and linker portions in protein-cell interactions at all.

Applicants solved the problems of low expression and aggregation by removing one or more of the amino acid residues in the amino terminal hydrophobic domain and/or linker moiety. Neither reference provide sufficient teaching or guidance as to the specific deletion of the amino acid residues that Applicants have discovered is necessary to overcome the problems of low yield and aggregation which occurred during the expression of the full length enzyme or the surprising result of increased catalytic activity and stability. The problems which Applicants have overcome, clearly are not appreciated in the teachings of Gilkes *et al.* and Halldorsdottir *et al.* That is, one of skill in the art upon reading the references alone or in combination would not appreciate that removal of these amino acids can provide a desirable increase in enzymatic activity and stability and overcome expression and aggregation problems. Since there is no appreciation in the references of the specific problems encountered and overcome by Applicants, there can be no solution provided.

To establish a *prima facie* case of obviousness, there must be a some suggestion within the references themselves to direct the skilled artisan to combine the art. As discussed in detail above, neither reference teaches the superior qualities of Applicants' claimed invention. Surprisingly, Applicants' enzyme has superior (*i.e.*, about three-fold higher) specific activity on carbon methyl cellulose than the full-length enzyme. Further, the deficiencies present in both references are not remedied by the high level of skill in the art. The results Applicants demonstrated are not suggested in any of the references and there would be no expectation of such a results from the teachings of the references. In view of the superior enzymatic properties obtained with the truncated protein, the cited references do not suggest Applicants' claimed

invention and therefore do not render obvious Applicants' invention. Reconsideration and withdrawal of the rejection are respectfully requested.

CONCLUSION

In summary, it is concluded that the art cited by the Examiner does not render obvious Applicants' claimed invention. Accordingly, reconsideration and withdrawal of the rejections are respectfully requested. Applicants' Agent respectfully requests a telephone conference with the Examiner before the issuance of a subsequent Office Action to further expedite the prosecution of this Application. Please call the undersigned or Alice O. Carroll, Esq. at the number given below.

Respectfully submitted,

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Concord, MA 01742-9133
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MARKED UP VERSION OF AMENDMENTS

1. (Amended) An isolated nucleic acid molecule encoding a polypeptide having improved thermostable cellulase activity and at least about 85% identity compared to SEQ ID NO: 2, [which] wherein [the polypeptide is a variant of a glycosyl hydrolasae of family 12, and wherein] said nucleic acid is truncated such that one or more of the amino acid residues corresponding to position one to about position 40 in the corresponding [full lengtht enzyme] SEQ ID NO: 2 are deleted in the polypeptide encoded by said nucleic acid molecule.

2. (Amended) A nucleic acid construct comprising the nucleic acid molecule of Claim 1 operably linked to a regulatory sequence.

3. (Amended) A host cell comprising the nucleic acid construct of Claim 2.

4. (Amended) An isolated nucleic acid molecule encoding a polypeptide having improved thermostable cellulase activity compared to its full-length native counterpart, wherein said polypeptide comprises the amino acid sequence of SEQ ID NO: 2, wherein one or more of the amino acid residues from position one to about position 40 are deleted.

5. (Amended) A nucleic acid construct comprising the nucleic acid molecule of Claim 4 operably linked to a regulatory sequence.

6. (Amended) A host cell comprising the nucleic acid construct of Claim 5.

7. (Amended) An isolated nucleic acid molecule encoding a polypeptide having improved thermostable cellulase activity compared to its full-length native counterpart, said nucleic acid molecule having a nucleotide sequence selected from the group consisting of: nucleotides 52-783 of SEQ ID NO: 3, nucleotides 55-783 of SEQ ID NO: 3, nucleotides 58-783 of SEQ ID NO: 3, nucleotides 61-783 of SEQ ID NO: 3, nucleotides 64-783 of SEQ

ID NO: 3, nucleotides 67-783 of SEQ ID NO: 3, nucleotides 70-783 of SEQ ID NO: 3, nucleotides 73-783 of SEQ ID NO: 3, nucleotides 76-783 of SEQ ID NO: 3, nucleotides 79-783 of SEQ ID NO: 3 and nucleotides 82-783 of SEQ ID NO: 3.

8. (Amended) A nucleic acid construct comprising the nucleic acid of Claim 7 operably linked to a regulatory sequence.

9. (Amended) A host cell comprising the nucleic acid construct of Claim 8.

10. (Amended) The isolated nucleic acid molecule of Claim 7 wherein the nucleic acid has the sequence of nucleotides 52-783 of SEQ ID NO: 3.

11. (Amended) An isolated nucleic acid molecule encoding a polypeptide having improved thermostable cellulase activity compared to its native full-length counterpart, said nucleic acid having a nucleotide sequence selected from the group consisting of: nucleotides 85-783 SEQ ID NO: 3,
nucleotides 88-783 of SEQ ID NO: 3, nucleotides 91-783 of SEQ ID NO: 3, nucleotides 94-783 of SEQ ID NO: 3, nucleotides 97-783 of SEQ ID NO: 3, nucleotides 100-783 of SEQ ID NO: 3, nucleotides 103-783 of SEQ ID NO: 3, nucleotides 106-783 of SEQ ID NO: 3, nucleotides 109-783 of SEQ ID NO: 3 and nucleotides 112-783 of SEQ ID NO: 3.

12. (Amended) A nucleic acid construct comprising the nucleic acid molecule of Claim 11 operably linked to a regulatory sequence.

13. (Amended) A host cell comprising the nucleic acid construct of Claim 12.

14. (Amended) The isolated nucleic acid molecule of Claim 11 wherein the nucleic acid sequence comprises the sequence of nucleotides 112-783 of SEQ ID NO: 3.

15. (Amended) An isolated nucleic acid molecule encoding a fusion protein comprising a thermostable cellulase having improved thermostable cellulase activity and at least about 85% identity compared to SEQ ID NO: 2 and a fusion partner, said thermostable cellulase is a variant of a glycosyl [hydrolysae] hydrolase of family 12, and wherein said nucleic acid molecule is truncated such that one or more of the amino acid residues corresponding to position one to about position 40 in [the corresponding full lengtht cellulase] SEQ ID NO: 2 are deleted in the fusion protein encoded by said nucleic acid molecule.

16. (Amended) The isolated nucleic acid molecule of claim 15 encoding a fusion protein comprising a thermostable cellulase and a fusion partner, said thermostable cellulase comprising the amino acid sequence of SEQ ID NO: 2 wherein said one or more of the amino acid residues from position one to about position 40 are deleted.

17. (Amended) A nucleic acid construct comprising the nucleic acid molecule of Claim 15 operably linked to a regulatory sequence.

18. (Amended) A host cell comprising the nucleic acid construct of Claim 17.

19. (Amended) A method for producing a thermostable cellulase comprising maintaining the host cell of Claim 18 under conditions suitable for expression of said nucleic acid construct, whereby said thermostable cellulase is produced.

20. (Amended) A method for producing a thermostable cellulase comprising maintaining the host cell of Claim 19 under conditions suitable for expression of said nucleic acid construct, whereby said thermostable cellulase is produced.